

the tip of the snout. Skin of the throat and sides of the body with minute tubercles. Reddish brown above, closely marbled with darker brown, tibiae with three dark cross-bars; pale beneath.

From snout to vent 35 mm.

Hab. Mount Saribaw, Samarahan River, Sarawak.

This rather obscure little frog in general appearance resembles small specimens of *Rana Kuhlîi*, D. & B., but the distinct tympanum and expanded tips of the digits readily serve to distinguish it from that species. The type and only known specimen (a female) is deposited in the Sarawak Museum.

XXV.—*The Heterogenetic Origin of Fungus-germs and Monads.* By H. CHARLTON BASTIAN, M.A., M.D., F.R.S., F.L.S.

[Plates XIV. & XV.]

SINCE my communication to 'Nature' on this subject, on Nov. 24 of last year, I have been devoting much of my leisure time to further observations on the development and transformations of small Zooglœa masses, with the result that I have abundantly confirmed the truth of my original observations, and have also been able to fill up several gaps in my previous knowledge. I have satisfied myself also that by far the best way for readily investigating these phenomena is to obtain very thin bacterial scums, by using filtered infusions not too strong, and a depth of fluid of rather less than one inch. As all the processes that I have been describing go on in the dark quite as well as in the light, the simplest plan is to filter the infusion, prepared as previously directed *, into small one-ounce earthenware pots, over which the covers are then placed till the time comes for the examination of their contents. If three or four pots are prepared at the same time, they may be opened at will on successive days, or some may be exposed to one temperature and some to another.

It is important to bear in mind two fairly distinct aspects of my observations, corresponding with different stages in the processes described. We have to do (1) with the growth, the individualization, and the processes of segmentation taking place in masses of Zooglœa. We have also to do (2) with

* 'Nature,' Nov. 24, 1904, p. 77.

the question of the ultimate destination, or the transformation, of the products of such segmentation. These are two parts of the subject that are to some extent distinct, and which are well worthy of further separate consideration.

(1) *The Developmental Tendencies in Zooglæa*.—If it be asked, what amount of knowledge do bacteriologists possess on this subject? the answer must be “next to nothing,” if we are to judge from the extreme paucity of information on the subject which is to be found in any of their text-books. Yet if they would only deign to look at what takes place in a filtered hay-infusion, they could very soon satisfy themselves that Zooglæa masses not only grow rapidly, but undergo definite developmental processes, associated with marked molecular changes, as evidenced by their different behaviour at different stages to logwood or other stains, as well as by the results of microscopical examination. And while these molecular changes are taking place the masses may segment into larger or smaller portions and often into minute spherical or ovoid units, showing that an organizing process is taking place.

If we look at the constitution of Zooglæa masses as shown in fig. 1, A and B (Pl. XIV.), it may be seen that we have only to do with bacteria imbedded in a varying proportion of glæal material. But later on, when the mass has grown, and some amount of segmentation has taken place, as in C, it may often be seen that we have still only to do with aggregates of bacteria. At other times, it is true, the molecular changes that have taken place in the mass have so altered its constitution (making the segments very refractive) that the included bacteria are no longer recognizable, as in the paler segments shown in D, which soon become resolved into the dark brown Fungus-germs seen by their side.

But now an important link in the proof of my views may be brought forward. The Zooglæa masses in their early stages are colourless, but a large proportion of them are, as I maintain, ultimately destined to give rise to brown Fungus-germs. The assumption of the brown colour may, however, be taken on by the segments of the Zooglæa while they are still only aggregates of bacteria, as may be seen by fig. 2, A, showing nearly spherical segments of a large Zooglæa mass which were in different shades of brown, and in some of which the constituent bacteria (now themselves coloured) are plainly to be seen. The same thing occurs not unfrequently in Zooglæa masses which have as yet undergone no segmentation, as in fig. 2, D. In B a small Zooglæa aggregate is seen becoming brown, the process being rather more advanced below

than above, while in C a similar mass is to be seen which has almost completely segmented into Fungus-germs. In E we have two other much smaller masses of Zooglœa which have become brown, and which plainly show their constituent bacteria; while in F we have the same process taking place in very minute units of Zooglœa, such as give rise to discrete Fungus-germs—though in other cases very similar minute units of Zooglœa, to which I shall presently refer, are transformed either into Monads or into Amœbæ.

These facts concerning the changes taking place in large or small masses of Zooglœa, even leaving aside the question of their subsequent transformations, are surely facts of considerable importance well worthy of a little attention on the part of bacteriologists.

Although my more complete recent observations as to the assumption of a brown colour by the Zooglœa masses and their segments serve to make the relationship between them and the brown Fungus-germs more obvious than it was previously, yet the actual proof of the development of the one from the other can now be given in a more complete way—also as a result of recent observations.

(2) *The Transformation of Zooglœa Masses into Fungus-germs or Monads.*—The very distinct specimens to which I shall first refer were taken from the scum on a hay-infusion that had been exposed in a small closed pot to a temperature of 70° F. (21° C.) for seven days. Many of the Zooglœa masses had by that time become wholly transformed into brown Fungus-germs, though other masses in all intermediate stages were to be seen. Some were still in an early colourless state, as in fig. 1, A, the mass there represented having been taken from this infusion. A later stage in which the whole mass is being converted into embryo Fungus-germs is shown in fig. 3, A; and a still later stage, in which the nucleated embryo germs are far more distinct, is shown in B. In C the germs may be seen to be larger and becoming brown, while in D (shown under a lower magnification) the entire mass of the Zooglœa has been transformed into aggregates of brown Fungus-germs.

In the pot from which these specimens were taken at the end of the seventh day not a single hypha was to be found, nor were there any of the thick-walled brown cells to which Mr. Massee refers*. All such bodies were similarly absent for many days after, during which from time to time I

* 'Nature,' Dec. 22, 1904, p. 175.

examined the contents of the pot. It was perfectly plain, indeed, that in the thousands of small Zooglœa masses undergoing this change, one had to do with no process of infection. It was clear that the Zooglœa masses were becoming organized simultaneously throughout their substance, and that all the stages of the development of the Fungus-germs into which they were being transformed could be more or less plainly traced, the changes in this case taking place without any antecedent minute segmentation of the masses. I invited Mr. Massee to come and examine these specimens for himself, but he did not do so, and nevertheless subsequently thought proper to write the letter already referred to, which appeared in 'Nature,' and was answered in a subsequent issue (January 19, 1905, p. 272).

A similar transformation of the Zooglœa masses without antecedent segmentation has been seen in other cases, though in none of them have I been able to make out the actual stages of the transformation anything like so plainly as in the specimens represented in fig. 3. In one of the cases recently seen, in which the specimens were also taken from a closed lot, the Zooglœa masses as a whole had previously assumed a pale brown tint, and rather large Fungus-germs were formed from their substance, such as are shown in fig. 4, A ($\times 700$). But, again, no hypha of any kind was ever seen among the contents taken from this pot.

Where a certain amount of segmentation has occurred in the Zooglœa mass before the brown colour is assumed (as in fig. 2, A, in which the constituent bacteria are still distinct), these brown segments subsequently become resolved into Fungus-germs, as may be seen in fig. 4, B. Such brown Fungus-germs soon assume more definite contours as in A, and they may occasionally, but very rarely, be seen to give rise to hyphæ.

As intimated in my previous communication to 'Nature,' there are various modes in which Fungus-germs originate from the Zooglœa masses. Still these different modes are only comparatively unimportant variations, dependent upon the different states of the Zooglœa masses at the time when the actual transformations take place. The principal variations seem to be these:—

(a) Zooglœa entire and uncoloured; organization throughout, and Fungus-germs when forming becoming brown, as in fig. 3.

(b) Zooglœa entire, but assuming a brown colour before the transformation into Fungus-germs occurs, as in fig. 2, D, and fig. 4, A.

(c) Zooglœa uncoloured, undergoing partial segmentation, and then the origination therefrom of Fungus-germs gradually becoming brown, as in figs. 8 and 9 in 'Nature' for Nov. 24th, 1904.

(d) Zooglœa uncoloured and undergoing partial segmentation; these segments then becoming brown, and subsequently being transformed into groups of brown Fungus-germs, as in figs. 2, A, and 4, B.

(e) A mixture of the last two processes, as shown to some extent in the lower part of fig. 9 ('Nature,' Nov. 24th).

(f) Zooglœa uncoloured and undergoing complete segmentation into colourless units, which gradually assume a brown or brownish-black tint, as in fig. 12 ('Nature,' Nov. 24th).

As I previously said, the conversion of the Zooglœa masses into aggregates of Monads takes place much less frequently than their transformation into Fungus-germs. Still the two processes may often be seen going on side by side in the pellicle. I am, indeed, disposed to think from what I have seen that some of the same kind of Zooglœa masses which in the early days become converted into Monads may, if they chance to remain untransformed for seven or eight days, be then converted into Fungus-germs rather than into Monads. It is difficult to be certain of this, but I am strongly disposed to believe that it is so. It is quite certain, however, that conversion into Monads, when it occurs, takes place almost always somewhere between the third and the fifth days, while after the latter date up to the tenth or twelfth day one finds Zooglœa masses either all brown or producing brown segments which are being converted into Fungus-germs. The Zooglœa masses and all the segments into which they divide invariably remain colourless where Monads or Amœbæ are to be the products.

Since my former communication to 'Nature' I have seen enormous numbers of the Zooglœa masses yielding Monads in the pellicle on an infusion of hay, which was exposed to light in a small beaker. The Monads were met with on the fourth and fifth days, the temperature to which the infusion had been exposed having varied from about 54°-59° F. (12°-15° C.). The Zooglœa masses were in many cases very large and presented some peculiar characters. In their early stages they always appeared as pellucid, somewhat ramified, discontinuous areas, as shown in fig. 5, A (Pl. XV.). Later, portions of the mass begin to segment as in B; later still, actual Monads may be seen beginning to form, as in fig. 6, A; while, finally, there is a more complete conversion into an aggregate of

motionless spherical or ovoidal units, as shown in fig. 6, B, which after a time become active flagellate Monads.

As I have indicated in my 'Studies in Heterogenesis,' pp. 69-73, Monads are much more commonly produced from the pellicle in another way—namely, as pale, discrete, *motionless* corpuscles more or less thickly distributed through its under layers. These are to be seen often in the course of the third or fourth day, when not a single active Monad has hitherto been found. While if the same pellicle be examined twelve or twenty-four hours later, the fluid may be discovered to be swarming with active Monads, all of about the same size, and this size agreeing with that of the previously motionless corpuscles. At other times swarms of minute Amœbæ rather than of Monads suddenly make their appearance where only motionless corpuscles were previously present.

As the corpuscles sometimes develop into Monads and sometimes into Amœbæ, I have been accustomed to speak of them as "indifferent corpuscles." Not unfrequently, however, after their formation they may remain for a much longer time quiescent and without developing in either direction. This was the case in a hay pellicle that I have recently examined, in which such corpuscles were found in enormous numbers and rather larger than usual. They were first seen when a small pot containing same hay-infusion was opened after four days, during which it had been exposed to a temperature of 70° F. (21° C.). The edge of a portion of this pellicle is represented in fig. 7, A, while in B some of the separate corpuscles are shown more highly magnified, so as to reveal the nature of their contents. Nothing like a nucleus is to be seen, nor can one be detected by the use of any of the ordinary stains even when the corpuscles have been allowed to soak in them for many hours. Logwood, carbo-fuchsine, gentian violet, and mastzellen stain have all yielded negative results. Not a trace of a nucleus is to be found, and the corpuscles seem to be mere individualized portions of Zooglœa intermediate in size between the brown units on the way to the production of Fungus-germs, which are shown in fig. 2, E and F, and, like them, containing only a few bacteria in their interior. I have examined such corpuscles over and over again, and always with similar results. If they do not speedily develop, a limiting membrane is produced which enables their contents to resist staining, and causes the corpuscles themselves to shrivel if they are mounted in glycerine and water. When these corpuscles develop quickly, which is the rule, they give rise, as I have said, more or less suddenly to swarms either of Monads or of minute

Amœbæ, and then, when thus developed, a delicate nucleus can generally be recognized even without the aid of stains.

Of course all this is very contrary to generally accepted beliefs; but if others would only investigate this subject with care, as I have done, I have little doubt but that my results would speedily be confirmed. The study of what goes on in the scum on a hay-infusion might, indeed, be strongly recommended to some who are occupied with researches on the origin of species, and the phenomena of variation in vegetal and animal organisms, as likely to yield more fundamental and more striking results than certain statistical and other methods of enquiry which at present find favour.

In conclusion, I would ask, Why do the bacteriologists not tell us what they know about Zooglœa—whether they are or are not aware of its developmental tendencies, and why it should undergo processes of minute segmentation, unless such processes are a result of an organizing tendency destined to have some definite outcome? Why, again, should it or its segments so often tend to assume a brown colour, while it is still nothing but Zooglœa, either segmented or unsegmented? Again, why, if the brown Zooglœa does not yield the brown Fungus-germs, should there be this constant association of myriads of brown Fungus-germs (in the absence of hyphæ) in association with brown masses of Zooglœa? How can they explain, other than I have done, the actual organization of a Zooglœa mass and the stages by which the brown Fungus-germs seem to be formed therein, such as are shown in fig. 3? What process of “infection” in a filtered hay-infusion contained in a closed pot could cause thousands of small Zooglœa masses to go simultaneously through similar processes of this kind—producing myriads of brown Fungus-germs—when not a single hypha is anywhere to be found, and where at first no Fungus-germs are to be met with outside the Zooglœa masses themselves? I trust the bacteriologists will vouchsafe to give us some information on these points, or, if they cannot reasonably explain them, that they may be induced to work at the subject, and satisfy themselves that something important can be learned concerning bacteria, even though it be outside their laboratories and by methods other than their own.

EXPLANATION OF THE PLATES.

PLATE XIV.

Fig. 1. A. An ordinary mass of Zooglœa in an early stage.

B. A more pellucid mass in which the gleeal material is unusually abundant.

Fig. 1. C. Portions of a large Zoogloea mass at a later stage in which the contained bacteria are still distinct, though altered, within the segments.

D. Glistening segments of a Zoogloea mass in which the bacteria are completely obscured, while by their side are other segments which have become resolved into brown Fungus-germs.

A, B, and D, $\times 500$; C, $\times 700$.

Fig. 2. A. Segments of a large Zoogloea mass becoming brown, in some of which the constituent bacteria may be seen.

B. A Zoogloea aggregate in an early stage becoming brown.

C. A similar mass from the same scum segmenting into pale brown Fungus-germs.

D. Zoogloea mass as a whole becoming brown.

E. Two small Zoogloea masses which have become of a darker brown colour.

F. Still smaller Zoogloea units which have become brown, and, like the last, also show the contained bacteria.

All $\times 500$.

Fig. 3. A. Portion of a Zoogloea mass which is beginning to be transformed into an aggregate of embryo Fungus-germs.

B. A later stage of the same change, in which the embryo germs are more distinct.

C. A later stage still, in which the germs are larger and becoming brown.

D. Masses of Zoogloea which have been completely transformed into aggregates of brown Fungus-germs.

A, B, and C, $\times 700$; D, $\times 500$.

Fig. 4. A. Zoogloea masses of a pale brown colour being transformed into very distinct Fungus-germs.

B. A Zoogloea mass converted into brown, roundish segments which are being transformed into Fungus-germs.

A, $\times 700$; B, $\times 500$.

PLATE XV.

Fig. 5. A. A pellucid ramifying mass of Zoogloea.

B. Commencement of segmentation in portions of such a pellucid mass.

Each $\times 500$.

Fig. 6. A. Early stage in the formation of Monads from a similar pellucid mass of Zoogloea.

B. More complete segmentation of such a Zoogloea mass into embryo Monads.

Each $\times 500$.

Fig. 7. A. Portion of a hay pellicle showing discrete motionless corpuscles scattered through it.

B. A few of these discrete corpuscles more highly magnified and showing contained bacteria but no nucleus.

A, $\times 500$; B, $\times 700$.